



Regular Article

Plant growth regulators alters antioxidant metabolisms in *Solanum trilobatum* L.: An underutilized medicinal herb

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Abstract

In this study, the changes in enzymatic and non-enzymatic antioxidants of *Solanum trilobatum* was estimated upon treatment with plant growth regulators (PGR) like Absciscic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA). The given treatments were started at 70th day followed by 80th, 90th and 100th Days After Sowing (DAS). The groups were treated with respect growth hormones by spraying method to ABA 10 $\mu\text{g L}^{-1}$, PBZ 10 mg L^{-1} and SA 500 $\mu\text{g L}^{-1}$ concentrations. The plant was harvested on 80th, 90th, 100th and 110th DAS and analyzed the non-enzymatic antioxidants like Reduced glutathione (GSH), Ascorbic acid (AA) and α -tocopherol contents and enzymatic antioxidants like Superoxide dismutase (SOD), Peroxidase (POX), Catalase (CAT) and Ascorbate peroxidase (APX) activities. In all the non-enzymatic and enzymatic antioxidant contents were increased by the growth regulators to a significant extent when compared with control plants. In our results indicated that the ABA, PBZ and SA applications at low concentration can be used as a potential tool to increase defence mechanisms in medicinal plants.

Key words:*Solanum trilobatum*, plant growth regulators, absciscic acid, paclobutrazol, salicylic acid, antioxidant activity**Received:**

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10.25081/jmb.2017.v1.51**Introduction**

Solanum trilobatum L. belongs to the family Solanaceae. It is commonly available in Southern India and it has been used in herbal medicine to treat various diseases like cough (Mihira et al., 2011) diabetes, vomiting with blood and leprosy (Doss et al., 2011). Besides these, it is also used to increase male fertility and also counter acts snake poison (Govindhan et al., 2004). *S. trilobatum* possess pharmacological properties viz., antiasthmatic (Govindhan et al., 1999), anticancer (Mohan and Devi, 1997) and antidandruff (Pant et al., 2013). The plant is also rich in phytochemical constituents like alkaloids, phenolics, flavonoids, sterols, saponins and glycosides (Amir and Kumar, 2004).

There are various studies on medicinal effects (Parasuraman et al., 2017; Kannan et al., 2016; Ahmed et al., 2016) and vegetable and edible aspects (Arinathan et al., 2007) but the effects of different plant growth regulators on this plant is still to be investigated.

Absciscic acid (ABA) as a plant growth regulators involved in many plant process like seed maturation, dormancy and adaptation to abiotic stresses (Beaudoin et al., 2000; Sreenivasulu et al., 2012). Paclobutrazol (PBZ) is a triazole group of systematic fungicide, and possesses regulating properties on plant growth (Fletcher and Arnold, 1986). The previous works were carried out in revealed that the

morphological and physiological changes associated with the triazole treatment in various plants, including the inhibition of the plant growth, decreased intermodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root and shoot ratio, increased antioxidant potentials, and an enhancement in alkaloid productions (Jaleel et al., 2007). Salicylic acid (SA) is an endogenous plant growth regulator (Metraux, 2002) and plays many significant actions in plants like plant growth, thermogenesis, flower induction, nutrient uptake, ethylene biosynthesis, stomatal movements, photosynthesis and enzyme activities (Hayat and Ahmed, 2007).

The objectives of the present investigation were conducted to evaluate the effects of PGR such as ABA, PBZ and SA on enzymatic and non-enzymatic antioxidant changes of *S. trilobatum* under field conditions.

Materials and methods

Solanum trilobatum economically important medicinal plant belongs to the family Solanaceae was selected for the present investigation. The seeds were obtained from the Horticulture Department in Annamalai University, Chidambaram in Tamil Nadu, India. Paclobutrazol is a triazolic group of fungicide having plant growth regulating properties obtained from CULTAR 25% w/v from Zeneca ICI Agrochemical Ltd., Mumbai, India. Absciscic acid and Salicylic acid were obtained from Himedia Mumbai.

Plant material and cultivation method

The field experiments were conducted at the Botanical Garden and Plant Growth Regulation Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India, and as explained by us (Nivedithadevi et al., 2012).

The seeds were surface sterilized with 0.2% mercuric chloride solution for 2 min and rinsed thoroughly with distilled water. The seeds were soaked for 3 hours in conical flask before sowing. The nursery bed is prepared with clay, red loam soil and Farm yard manure (FYM) in 1:1:1 ratio. Then seeds were spread on the nursery bed. The plants were allowed to grow till 40 day with regular irrigation. The seedlings were selected with 10-12cm height and develop 6 leaves for even growth conditions.

The field is laid out exactly as for ridged, irrigated sufficiently and, after ploughing twice, is watered heavily and ploughed again. FYM and neem cake will give as fertilizers. In the initial period, irrigation is done once in a week and then in later stages as per requirement. The selected plants were transplanted to field. After 70 day the treatments like PBZ at 10 mg L⁻¹, ABA at 10 µg L⁻¹ and SA at 500 µg L⁻¹ were started at 70th day followed by 80th, 90th and 100th days. The groups were treated with respect growth hormones by spraying method. After every treatment of the 10th day, the plants were harvested for further analysis.

Determination of non-enzymatic antioxidants

The reduced glutathione content was assayed as described by Griffith and Meister (1979) and expressed reduced glutathione content in µg g⁻¹ fresh weight (FW). Ascorbic acid content was assayed as described by Omaye et al. (1979) and the results were expressed in mg g⁻¹ dry weight (DW). α-tocopherol content was assayed as described by Backer et al. (1980) and expressed in mg g⁻¹ fresh weight (FW).

Determination of enzymatic antioxidant activities

The peroxidase activity was determined by the method of Reddy et al. (1995). One unit (U) of peroxidase is defined as the change in absorbance/min. at 430 nm. Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich (1971). SOD activity was expressed in units. One unit (U) is defined as the amount of change in the absorbance by 0.1 h⁻¹ mg⁻¹ protein. The Ascorbic peroxidase activity was determined by the method of Asada and Takahashi (1987) and expressed in U mg⁻¹ protein. The catalase activity was measured according to the method of Chandless and Scandalios (1984). The enzyme activity is expressed in U mg⁻¹ protein. (U = 1 mM of H₂O₂ reduction min⁻¹ mg⁻¹ protein).

Results

Non-enzymatic antioxidant contents

The reduced glutathione content was increased all the growth regulators treated plants when compared to control. At 110 DAS PBZ treated plants GSH content was highest level increased on 430.87 per cent over control as

followed by ABA (347.43%) and SA (324.19%). Among the treatments the highest GSH content was recorded in PBZ followed by ABA and SA in all sampling days.

The ascorbic acid content of the growth regulator treated *S. trilobatum* plant was increased in all the sampling days when compared to control. The plant ascorbic acid content of leaves and it was 414.60, 319.10 and 304.49 per cent over control respectively in PBZ, ABA and SA treated plant on 110 DAS. Among the treatments PBZ showed the higher ascorbic acid content.

In leaves of *S. trilobatum* plant α -tocopherol content was increased by the growth regulator treated plant at all sampling days. Among the treatment PBZ highly increased the α -tocopherol content and it was 186.44 per cent over control followed by ABA 162.63 and SA 145.78 per cent over control and on 110 DAS.

Antioxidant enzymes activities

The peroxidase content of the growth regulator treated *S. trilobatum* plant was increased in all the sampling days when compared to control. Peroxidase content was greater than 211.80 per cent in the ABA treated plants and also PBZ and SA reasonably increased the peroxidase activity was 180.74 and 168.94 per

cent over control plants on 110 DAS. Among the treatments the highest peroxidase content was recorded in ABA followed by PBZ and SA in all sampling days.

In all the sampling days the SOD activity was increased when compared to control. The 110 DAS ABA treated plant recorded highest SOD content was found in 189.01 per cent, followed by PBZ (158.58 %) and SA (133.40%) over control plants. Among the treatments the highest SOD content was recorded in ABA followed by PBZ and SA in all sampling days.

Ascorbate peroxidase activity was increased in all the treatment plants when compared to control. In 110 DAS the ABA treated plants 227.16 per cent followed by PBZ and SA treated plants showed the minimal increased as 218.96 and 174.23 per cent over control respectively. Among the treatments the highest ascorbate peroxidase activity was recorded in ABA followed by PBZ and SA in all sampling days.

When compared with control plants, the increased CAT activity was found to be 275.72 per cent in ABA treated plants, followed by PBZ in 215.22 and SA 200.41 per cent over control for 110 DAS. The highest catalase activity was recorded in ABA followed by PBZ and SA in all sampling days.

Table 1. Effect of different growth regulators on antioxidant variations of *S. trilobatum* in different growth stages.

DAS	Control	ABA	PBZ	SA
Reduced Glutathione				
80	0.505 \pm 0.02 ^a	1.520 \pm 0.01 ^c	1.917 \pm 0.01 ^d	1.145 \pm 0.01 ^b
90	0.635 \pm 0.02 ^a	1.955 \pm 0.01 ^c	2.485 \pm 0.01 ^d	1.735 \pm 0.03 ^b
100	0.755 \pm 0.04 ^a	2.542 \pm 0.02 ^c	3.090 \pm 0.02 ^d	2.282 \pm 0.02 ^b
110	0.839 \pm 0.02 ^a	2.915 \pm 0.12 ^c	3.615 \pm 0.01 ^d	2.720 \pm 0.21 ^b
Ascorbic acid				
80	1.06 \pm 1.34 ^a	3.04 \pm 2.60 ^c	3.80 \pm 2.59 ^d	2.76 \pm 5.54 ^b
90	1.32 \pm 1.95 ^a	3.88 \pm 1.88 ^c	4.98 \pm 1.65 ^d	3.67 \pm 2.18 ^b
100	1.54 \pm 1.67 ^a	4.72 \pm 2.54 ^c	6.18 \pm 2.11 ^d	4.58 \pm 3.41 ^b
110	1.78 \pm 2.39 ^a	5.68 \pm 4.48 ^c	7.38 \pm 3.16 ^b	5.42 \pm 9.55 ^d
α -tocopherol				
80	2.02 \pm 0.20 ^a	2.71 \pm 0.54 ^c	3.27 \pm 0.11 ^d	2.22 \pm 0.07 ^b
90	2.26 \pm 0.36 ^a	3.34 \pm 0.24 ^c	3.89 \pm 0.06 ^d	2.72 \pm 0.56 ^b
100	2.52 \pm 0.38 ^a	3.89 \pm 0.11 ^c	4.56 \pm 0.04 ^d	3.35 \pm 0.04 ^b
110	2.73 \pm 0.55 ^a	4.45 \pm 0.06 ^c	5.09 \pm 0.01 ^d	3.98 \pm 0.70 ^b

Values are given as mean \pm SD. of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $P \leq 0.05$ (DMRT).

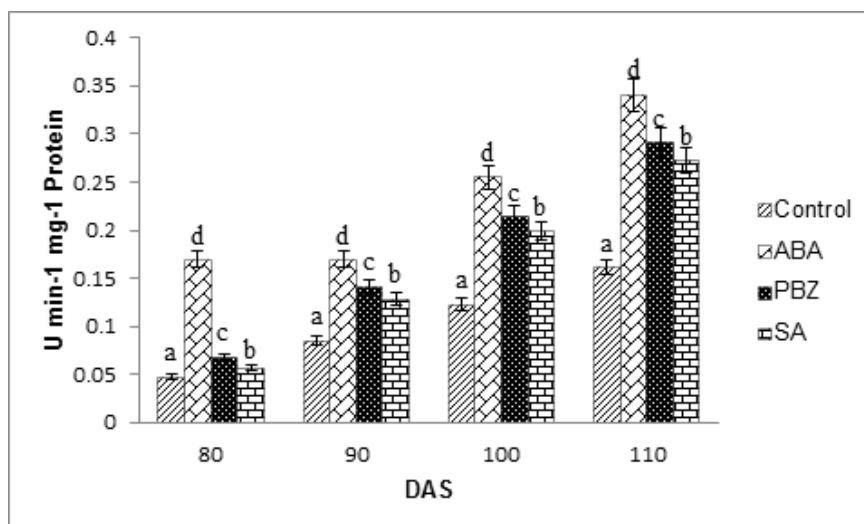


Fig. 1. Effect of different growth regulators treatments on the peroxidase activity in *S. trilobatum* on different growth stages. Values are given as mean \pm SD. of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $P \leq 0.05$ (DMRT).

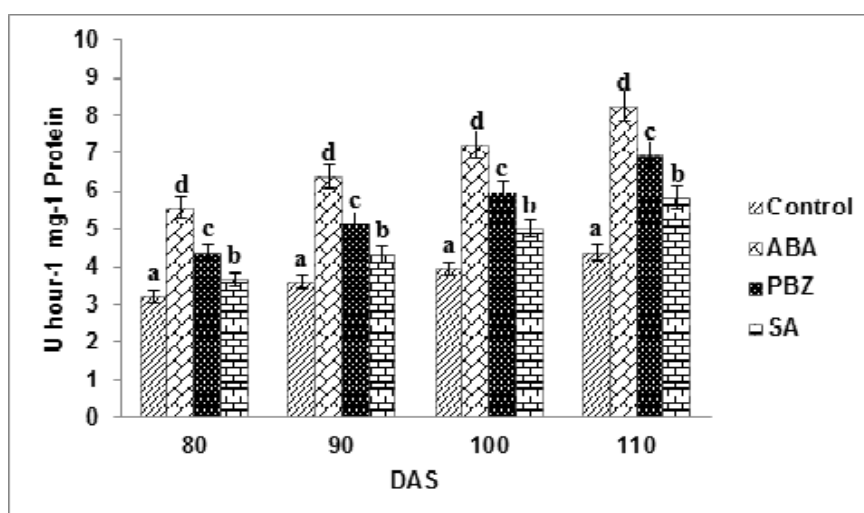


Fig. 2. Effect of different growth regulators treatments on the superoxide dismutase activity in *S. trilobatum* on different growth stages. Values are given as mean \pm SD. of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $P \leq 0.05$ (DMRT).

Discussion

The GSH content of the plant increased with the age in control and growth regulators treated *S. trilobatum*. All the three growth regulators were increased the GSH content significantly when compared to control. The similar report was observed at paclobutrazol (Sivakumar and

Panneerselvam, 2011) in *Datura metal*. SA pre-treatment significantly accelerated the accumulation of glutathione content in wheat (Kang et al., 2013). Our results also show that application of SA significantly increases the content reduced glutathione content.

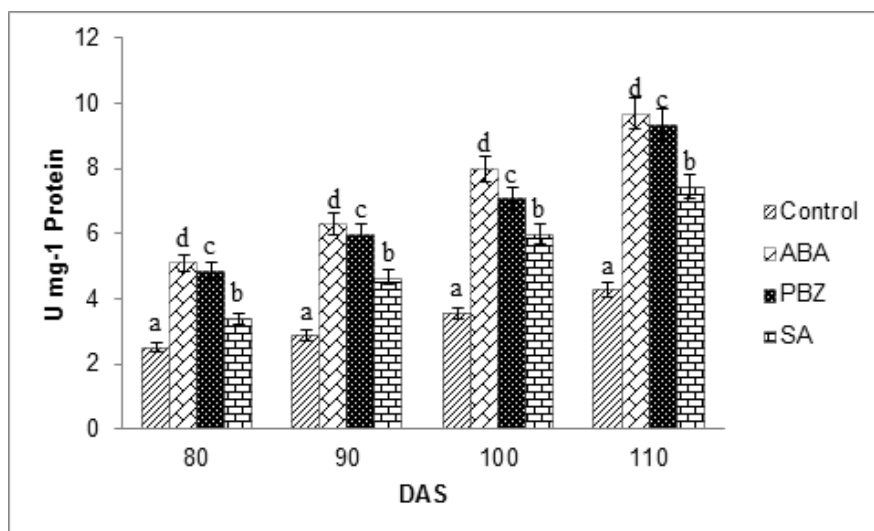


Fig. 3. Effect of different growth regulators treatments on the ascorbic peroxidase activity in *S. trilobatum* on different growth stages. Values are given as mean \pm SD. of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $P \leq 0.05$ (DMRT).

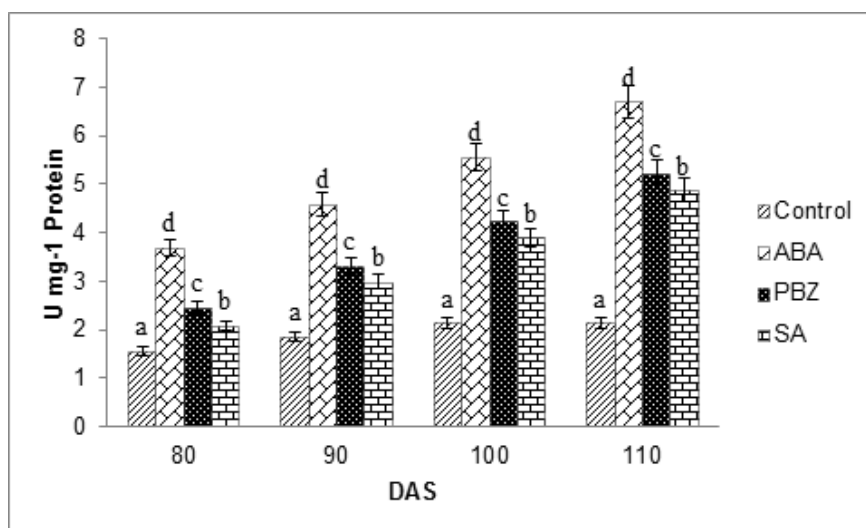


Fig. 4. Effect of different growth regulators treatments on the catalase activity in *S. trilobatum* on different growth stages. Values are given as mean \pm SD. of six samples in each group. Bar values are not sharing a common superscript (a, b, c, d) differ significantly at $P \leq 0.05$ (DMRT).

AA acts as an antioxidant, protecting cells against oxidative stress. AA is found to be one of the best characterized compounds, required for many key metabolic functions in plant cells (Smirnoff and Wheeler, 2000). In the present investigation, it was observed that growth regulators treated *S. trilobatum* plant increased AA content when compared to control plants. Similar findings were made in ABA increased (Guo et al., 2012). Increase in ascorbic acid

content was reported in the triazole treated *Helianthus annuus* and *Vigna unguiculata* (Manivannan et al., 2008). Then SA pre-treatment significantly accelerated the accumulation of GSH and AA in eggplant (Chen et al., 2011).

The growth regulator treatment to increase α -tocopherol content of the *S. trilobatum* plant when compared to control. Similar observations were made ABA increases in *Lactuca sativa* (Al

Muhairi et al., 2015). Then triazole increased the level of antioxidant ascorbic acid and α -tocopherol like in tomato seedlings and *Helianthus annuus* protected the membrane by preventing or reducing oxidative damage (Amalan Rabert et al., 2013). The SA treatments increased the α -tocopherol content from other worker (Munne-Bosch and Penuelas, 2003).

The SOD activity of the *S. trilobatum* was increased on various growth regulating treatments when compared to control. Similar to our results, some report has showed the growth regulator to induce an increase SOD activity. ABA increases the activities of antioxidant enzymes on two previous studies, such as SOD, catalase, APX and glutathione reductase in plant tissues under drought and freezing stress (Bano et al., 2012; Yang et al., 2013). The results are similar to those of triazole treatments in *Capsicum annuum* (Amalan Rabert et al., 2013). There are data supporting the claim that SA increases the activities of antioxidant enzymes (Ali Ghasemzadeh and Hawa, 2013; Pirasteh Anosheh et al., 2012).

In the present study growth regulators treatment results in *S. trilobatum* was increase of the APX activity when compared to control. Results of present investigation are consistence with the findings of ABA increased the APX content in (Zhang et al., 2014). Triazole treatment increased APX activity when compared to the case of control and drought-stressed plants (Manivannan et al., 2007; Amalan Rabert et al., 2014). Increasing APX activity as a consequence of exogenous SA application was also reported by (Krantev et al., 2008; Mishra et al., 2013).

The CAT activity was increased all the growth regulator treatments when compared to control. Similar results were obtained by many workers in many higher plants under ABA, PCZ and SA treated plants. ABA increased the CAT activity was observed by (Divya Nair et al., 2009) in *Ocimum sanctum*. The triazole treatments an increase in catalase activity was reported in higher plants when compared to control plants were noted in tomato and *Solenostemon rotundifolius* (Mohamadi and Rajaei, 2013; Kishorekumar et al., 2008). SA application was also increased in CAT and POX in heat stress (Kaur et al., 2009; Orabi et al., 2010).

The *S. trilobatum* plant POX activity was increased significantly at growth regulator treatments when compared to control. These results are in conform to the observations carried out by (Basak et al., 2012) in ABA treatment. Then activities of POX have been increased by the triazole application observed in *Withania somnifera* (Jaleel et al., 2008; Hojati et al., 2011). Then SA treatment increased the POX activities when compared to control this results agree with (Chen et al., 2014) in *Zoysia japonica*.

Conclusion

From the results of this investigation, it is clear that, the growth regulators ABA, PBZ and SA treatments were increased the AA, α -tocopherol and GSH of *S. trilobatum*. Antioxidant enzymes like, APX, SOD, POX and CAT were increased using the above treatments in *S. trilobatum*. In conclusion, our results indicated that the ABA, PBZ and SA applications at low concentration can be used as a potential tool to increase defense mechanisms in medicinal plants.

Author contributions

D.N and M.A worked under the supervision of R.S. All authors participated in the writing of the manuscript. All the authors agreed the final version of manuscript for publishing in Journal of Medicinal Botany.

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